Brit. J. Pharmacol. (1959), **14**, 467.

THE SCHISTOSOMICIDAL AND TOXIC EFFECTS OF SOME *N*-*p*-AMINOPHENOXYALKYLAMIDES

BY

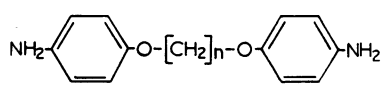
R. F. COLLINS, M. DAVIS, N. D. EDGE, J. HILL, H. W. READING, AND ELEANOR R. TURNBULL*

From the Research Laboratories, May & Baker Ltd., Dagenham, Essex

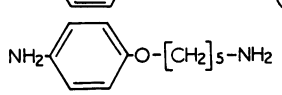
(RECEIVED MAY 7, 1959)

Several *N*-(ω-*p*-aminophenoxyalkyl)amides were active against *Schistosoma mansoni* in mice. One of the most effective, *N*-(5-*p*-aminophenoxypentyl)benzamide (M&B3002), acted more rapidly than lucanthone on adult worms but less rapidly than antimony potassium tartrate. It was inactive against immature worms. This compound and M&B2948A (*N*-(5-*p*-aminophenoxypentyl)phthalimide) were both active against *S. mansoni* in hamsters. In monkeys M&B2948A was inactive, whilst M&B3002 was not tested for therapeutic activity. Several of the compounds were examined for the production of visual impairment in cats. Although this property was not entirely absent, its incidence was very much lower in this amide series than among other ω-*p*-aminophenoxyalkyl derivatives not containing an amide group. M&B3002 and M&B2948A produced impairment of vision in only a small proportion of the large number of cats tested. The general toxicology of the two drugs was studied in several species, and also their absorption and excretion in mice and rats; this was to provide information for a clinical trial.

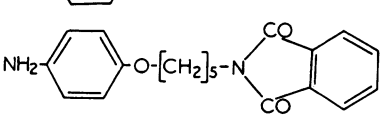
Following the discovery that a number of αω-di(*p*-aminophenoxy)alkanes (I) and related monoamines (Raison and Standen, 1955; Caldwell and Standen, 1956; Collins, Davis, Edge, and Hill, 1958) were active against *Schistosoma mansoni* in mice, we prepared and tested other ω-*p*-aminophenoxyalkyl derivatives, one of which was the dimethanesulphonate of 5-*p*-aminophenoxypentylamine (1-*p*-aminophenoxy-5-aminopentane) (II). This compound and (5-*p*-aminophenoxypentyl)-dimethylamine and -cyclohexylamine were inactive against *S. mansoni* and free from retinotoxic effects, but the intermediate for the preparation of 5-*p*-aminophenoxypentylamine, namely *N*-(5-*p*-aminophenoxypentyl)phthalimide, (M&B2948A) (III), showed marked activity.



(I)



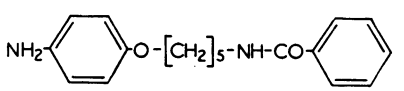
(II)



(III)

*Present address: Department of Zoology, University of Glasgow.

The corresponding benzamide (M&B3002) (IV) was then prepared and found to be active. Many other amides and imides were made subsequently, and this series forms the subject of the present paper.



(IV)

As in the previous investigation (Collins *et al.*, 1958) some of these compounds were examined for retinotoxicity in cats; this is known to be a potential hazard with substances of this type (Edge, Mason, Wien, and Ashton, 1956; Ashton, 1957; Goodwin, Richards, and Udall, 1957; Sorsby and Nakajima, 1958). Although some effect was found in a few of the cats given M&B2948A or M&B3002, it was much less common among the amides and imides than among the earlier compounds. The former were therefore potentially more useful and safer for administration to man, and preliminary clinical trials carried out by Alves, Harper, and Hill (unpublished observations) showed that M&B2948A, when given to children with *S. haematobium* infections, was effective and had no deleterious action on the eyes.

METHODS

Schistosomicidal Activity

The methods employed have been fully described by Hill (1956) and Collins *et al.* (1958). Groups of 12 to 15 mice infected with adult *S. mansoni* were given oral doses daily for four days. They were killed and examined for worms one to two weeks after the end of treatment. In the preliminary screening, test compounds were considered to be active if the number of living worms recovered from the mesenteric veins, portal vein, and liver was significantly fewer (t test, $P<0.01$) than in untreated controls. Many of the active compounds were examined further, using groups of 12 to 15 mice for each dose, to determine the CD50. The LD50 after a single dose was found for each compound, using five mice with each dose, and some of the active drugs were examined further for chronic toxicity by giving four daily doses to groups of 10 mice.

Two of the compounds (M&B2948A and M&B3002) were investigated in more detail than the others. With M&B3002 various dosing schedules were studied, as were the rate of action (Hill, 1956) and the effect on different developmental stages of the worm. Both compounds were also tested for activity against *S. mansoni* in hamsters, and M&B2948A in monkeys. In all these experiments the compounds were given by mouth.

Toxic Effects

Several of the compounds were administered to cats to see if they produced the weakening of the light and blink reflexes which are early signs of visual impairment (Collins *et al.*, 1958). Usually each compound was given to two cats in single oral doses of about 0.4 g./kg. of body weight, but three of the substances were administered to a larger number of cats using higher single and/or multiple doses. The general toxicology of M&B2948A and M&B3002 was examined in several species, the compounds being given orally unless otherwise stated.

Biochemical Estimations

Calibration curves were constructed by adding known quantities of the compounds to blood, urine, and faeces, and assaying the drug.

The methods of estimation all depend on the diazotization of the aromatic amino group of the compounds, followed by reaction with the coupling reagent, *N*-β-sulphatoethyl-*m*-toluidine (Rose and Bevan, 1944). This reagent produced azo dyes with the diazotized compounds with an absorption maximum at 520 mμ. After diazotization and coupling, a few ml. of absolute ethanol were added to the tubes to completely dissolve the azo dyes.

Drug concentrations were estimated as "free" and "total" material. "Free" drug represents diazotizable material prior to hydrolysis whilst "total" drug is that present after hydrolysis. It was always necessary to include "blanks" from biological material containing no drug.

Blood.—Concentration of free drug in blood samples was estimated in protein-free filtrates whereas total drug was estimated in similar filtrates after acid hydrolysis.

Urine.—Free drug was estimated by suitably diluting urine with dilute HCl and diazotizing without further treatment. Similarly, total drug was estimated after acid hydrolysis.

Faeces.—Extracts of faeces were prepared either by grinding with sand or by maceration in the presence of a warm aqueous solution (15%) of trichloracetic acid. The extract was filtered through a pad of Supercel in a small Buchner funnel. Drug estimations were carried out on the clear filtrate as described for urine.

RESULTS

Screening Experiments

Schistosomicidal Activity.—Among the aliphatic amides listed in Table I, the most active were the trichloracetamide (M&B4433) and the *p*-chlorophenoxyacetamide derivatives (M&B3435). The former was more toxic to mice and the latter was not more active than the benzamide derivative (M&B3002, Table II). The hippuramide (M&B3637) was less effective than these two compounds, and it had a very low cumulative toxicity to mice.

M&B3002 was one of the most potent of the benzamides (Table II). Substitution in the amino group of M&B3002 (Table III), other than by a single methyl group, decreased activity.

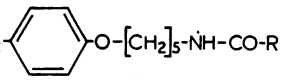
Table IV gives the results with a homologous series of phthalimides. The pentane to the nonane members were effective, although the last was much less so than the others. Isomers of M&B2948A in which the amino group was *ortho* or *meta* to the ether link were inactive, as was also an analogue which had no amino group. Substitution in the amino group of M&B2948A (Table V) reduced activity in all the types examined with the exception of M&B3430.

All the compounds in Tables VI and VII were less active than M&B2948A with the exception of the succinimide (M&B3023), the thenoylamide (M&B3124) and the piperidone (M&B3414), each of which was, however, more toxic than M&B2948A.

Ocular Effects.—None of the compounds produced ocular effects during the preliminary screening test, but M&B2948A, M&B3002, and M&B3637 affected the retinae of a few cats when more extensive examinations were carried out. M&B3012 (Table I) caused shivering, salivation, and repeated vomiting in cats.

TABLE I

ALIPHATIC AND SUBSTITUTED ALIPHATIC AMIDES

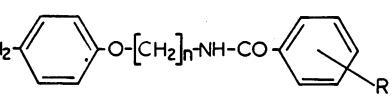
Group formula: 

All compounds were tested as the base except those marked with an asterisk, which were tested as the methanesulphonate. †, positive ocular response in only a few cats (Table VIII); ‡, no ocular response in cats. The following compounds were also therapeutically inactive: *N*-5-*p*-aminophenoxypentyl-*N*-phenylacetamide and 1-(5-*p*-aminophenoxypentyl)-3-*p*-ethoxyphenylthiourea.

M&B No.	–R	LD50 (g./kg.) Single Dose	LD50 (g./kg.) Daily Dose	CD50 Daily Dose (g./kg.)
4434	–H	0·75		>0·20
3012	$-CH_3$	1·5	0·80	0·42‡
4319	$-C_2H_5$	1·0		>0·40
4312	$-[CH_2]_4-CH_3$	>4·0		Inactive
4253	$-CHCl_2$	1·2		0·40
4433	$-CCl_3$	0·50		0·20
4454	$-CH_2CN$	2·5		<0·80
3180	$-CH_2-C_6H_5$	1·0		>0·30
3236	$-[CH_2]_4-C_6H_5$	4·0		Inactive
3208	$-CH=CH-C_6H_5$	>4·0		>1·0
4410	$-CH(C_6H_5)_2$	>4·0		<1·0
3386	$-CH_2-O-C_6H_5$	2·0		>0·60
3435	$-CH_2-O-C_6H_4-Cl-p$	>4·0		0·20
3584	$-CH_2-O-C_6H_3-Cl_2-2,4$	>4·0		>0·20
3751	$(\pm)-[CH_2]_2-NH-CO-CH(OH)-C(CH_3)_2-CH_2OH$	>1·0		>0·50
3637	$-CH_2-NH-CO-C_6H_5$	>4·0	3·0	0·38†
3707	Phthalimidomethyl	>4·0		>1·0
3237	Cyclohexyl	>4·0		Inactive
3169	$-OC_2H_5$*	1·0		>0·3
4448	$-CO-OC_2H_5$*	0·75		Inactive
3166	$-NH_2$	1·5		,,
4381	$-NH-[CH_2]_5-O-C_6H_4-NH_2-p$	>4·0		,,
4349	$-CO-NH-[CH_2]_5-O-C_6H_4-NH_2-p$	>4·0		>1·0
3241	$-[CH_2]_3-CO-NH-[CH_2]_5-O-C_6H_4-NH_2-p$*	3·0		Inactive

TABLE II

BENZAMIDES

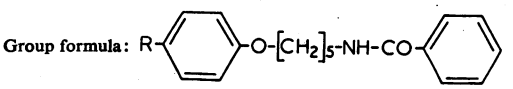
Group formula: 

All compounds were tested as the base except that marked with an asterisk, which was tested as the methanesulphonate. †, Positive ocular response in only a few cats (Table VIII); ‡, no ocular response in cats. *N*-(5-*p*-aminophenoxy-1-phenylpentyl)benzamide was also therapeutically inactive.

M&B No.	n	–R	LD50 (g./kg.) Single Dose	LD50 (g./kg.) Daily Dose	CD50 Daily Dose (g./kg.)
3210	4	–H	3·0		Inactive
3002	5	–H	>4·0	0·88	0·20†
3189	8	–H	4·0	0·57	0·16‡
4536	5	$p-NO_2$	>4·0		<1·0
3104	5	$p-NH_2$*	1·0		Inactive
3694	5	$p-NH-CO-CH_3$	>4·0		>0·20
3196	5	p-Cl	>4·0		>1·0
4422	5	p-Br	>4·0		1·0
3194	5	$p-CH_3$	>4·0		Inactive
3629	5	o-OH	>4·0		,,
3541	5	p-OH	1·0		,,
3487	5	$o-OCH_3$	3·0		>0·20
3439	5	$m-OCH_3$	1·5		0·20
3161	5	$p-OCH_3$	>4·0	0·69	0·29‡
3373	5	$o-CO_2Li$	3·0		>0·80
4308	5	$p-CO_2H$	>4·0		Inactive
4260	5	$p-CO_2CH_3$	1·5		,,

TABLE III

BENZAMIDES, SUBSTITUTED IN THE AMINO GROUP

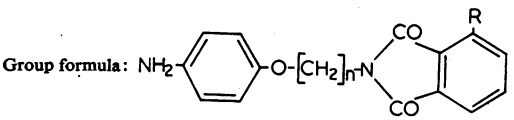
Group formula: 

All compounds were tested as the base.

M&B No.	–R	LD50 (g./kg.) Single Dose	LD50 (g./kg.) Daily Dose	CD50 Daily Dose (g./kg.)
3670	$-NHCH_3$	>4·0	1·0	0·20
3300	$-N(CH_3)_2$	>4·0	0·83	0·50
3556	$-NH-CH_2-CH_2OH$	4·0		0·40
3199	$-N(CH_2-CH_2OH)_2$	>4·0	1·8	>0·80
3572	$-NH-CH_2-CH_2-OCH_3$	2·0		>0·60
3549	$-NH-CH_2-CH(OH)-CH_2OH$	>4·0		>1·0
3552	$-N[CH_2-CH(OH)-CH_2OH]_2$	>4·0		Inactive
3561	$-N[CH_2-CH(OH)-CH_3]_2$	>4·0		<1·0
3380	$-NH-CH_2-CO-NH_2$	>4·0		Inactive
3416	$-NH-CH_2-SO_3Na$	4·0		>0·20
3385	$-NH-CO-CH_3$	>4·0		Inactive
3734	D-Glucosylamino	>4·0		>0·40

TABLE IV

PHTHALIMIDES

Group formula: 

All compounds were tested as the base except those marked with an asterisk, which were tested as the methanesulphonate. †, Positive ocular response in only a few cats (Table VIII); ‡, the corresponding hex-3-ene, tested as the methanesulphonate, had an LD50 (daily dose) of 1·0 mg./g. and a CD50>0·20 mg./g. The following compounds were also therapeutically inactive: *N*-(5-*p*-aminophenoxy-3-methylpentyl)phthalimide, *N*-(5-*p*-aminophenoxy-1-phenylpentyl)phthalimide and *N*-(5-*p*-aminophenoxypentyl)tetrachlorophthalimide.

M&B No.	n	–R	LD50 (g./kg.) Single Dose	LD50 (g./kg.) Daily Dose	CD50 Daily Dose (g./kg.)
3155	2	–H*	0·8		Inactive
3058	3	–H*	1·5		,,
3192	4	–H	>4·0		,,
2948A	5	–H	>4·0	1·02	0·18†
3153	6‡	–H	>4·0	1·24	0·15
3215	7	–H	4·0		0·14
3167	8	–H	>4·0		0·22
3187	9	–H	>4·0		>1·0
3291	10	–H	>4·0		Inactive
3693	5	$-NO_2$	1·5		,,
3752	5	$-NH_2$	>4·0		,,

Selection of Compounds for Further Study

M&B2948A and M&B3002, the first compounds of this type to be tested in mice, showed equal activity to lucanthone, but had lower chronic toxicity.

	CD50 (g./kg./day)	LD50 (g./kg./day)
Lucanthone	0.19	0.18
M&B2948A	0.15	1.20
M&B3002	0.20	0.88

Because of this and since at the time they were thought to be free from the ocular effects associated with some related compounds (Goodwin *et al.*, 1957; Collins *et al.*, 1958), further toxicity and therapeutic experiments were carried out to provide information for a clinical trial.

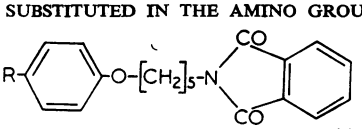
Later, M&B3637 (Table I) was found to be less toxic than M&B2948A and M&B3002 on repeated administration to mice, and, since the last two compounds were then known to produce ocular effects in a few cats, M&B3637 was also given to several cats (Table VIII) to see if it was completely free from these effects. However, no further experiments were done when it was found also to produce mydriasis and especially since it was less active in mice than M&B2948A and M&B3002. The hexane and heptane homologues of M&B2948A (Table IV) were not sufficiently more active than M&B2948A to warrant further study, particularly since the intermediate compounds were slightly less accessible chemically. None of the other compounds showed any advantage over M&B2948A and M&B3002 in the screening tests.

Results with M&B2948A and M&B3002

Schistosomicidal Activity. — Two rhesus monkeys and one green monkey (*Cercopithecus*

TABLE V

PHTHALIMIDES, SUBSTITUTED IN THE AMINO GROUP

Group formula: 

All compounds were tested as the base. The LD50 marked with an asterisk was 0·8 g./kg. when the drug was given in daily doses.

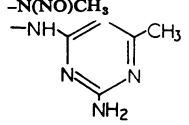
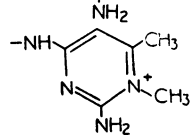
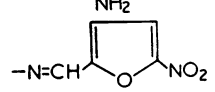
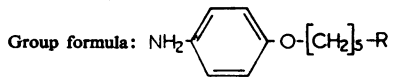
M&B No.	-R	LD50 Single Dose (g./kg.)	CD50 Daily Dose (g./kg.)
3413	$-NHCH_3$	>4·0	>0·20
3430	$-N(CH_3)_2$	4·0	0·23
3031	$-N(CH_2-CH_2OH)_2$	>4·0*	>0·40
4202	$-N(CH_2-CH_2Cl)_2$	>4·0	Inactive
3782	$-NH-CO[CH_2]_2-CO_2NH_4$	4·0	,,
4186	$-N(NO)CH_3$	>4·0	,,
3763		>4·0	>1·0
3819		>4·0	Inactive
4396		>4·0	,,

TABLE VI

OTHER IMIDES

Group formula: 

All compounds were tested as the base except that marked with an asterisk (as the methanesulphonate) and that marked † (as the sulphate). ‡, No ocular response in cats; §, LD50 (daily dose) =0·5 g./kg.

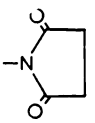
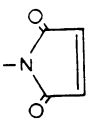
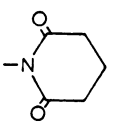
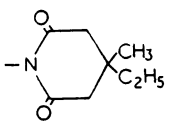
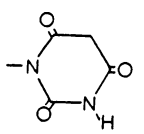
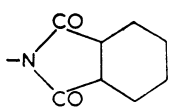
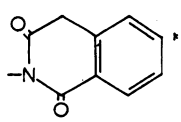
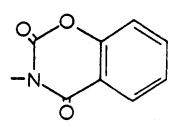
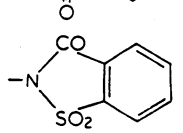
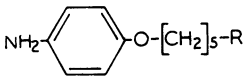
M&B No.	-R	LD50 Single Dose (g./kg.)	CD50 Daily Dose (g./kg.)
3023		3·5§	0·19‡
4310		1·5	>0·40
3885		3·0	>0·40
3952		>4·0	>0·20
3741		>4·0	Inactive
3429		>4·0	0·30
3586		>4·0	>1·0
3461		>4·0	>0·20
3105		>4·0	>0·20‡
4562	(±)-Camphorimido†	4·0	<0·80
3495	$-N(CO-CH_3)_2$	3·0	0·80
3718	$-N(CO-C_6H_5)_2$	>4·0	>1·0

TABLE VII
OTHER AROMATIC CARBOXYAMIDES

Group formula: 

All the compounds were tested as the base except that marked with an asterisk, which was tested as the methanesulphonate.

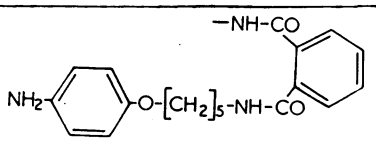
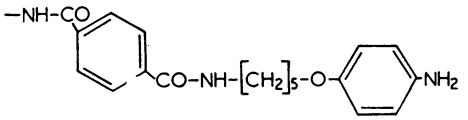
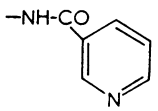
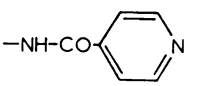
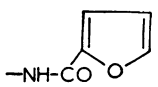
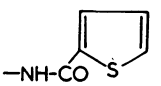
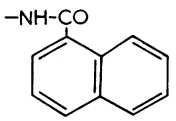
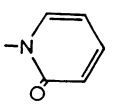
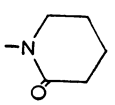
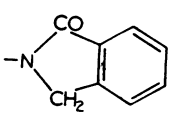
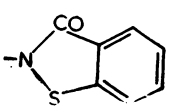
M&B No.	–R	LD50 (g./kg.)		CD50 Daily Dose (g./kg.)
		Single Dose	Daily Dose	
3614		>4·0		>1·0
4262				Inactive
3181		1·0	0·35	>0·20
3207		1·5		>0·40
3212		3·0	0·45	>0·45
3214		3·0	0·28	0·21
3209		>4·0	0·60	0·43
3205	–N(CH$_3$)–CO–C$_6$H$_5$	0·75		>0·25
3137	–N(C$_6$H$_5$)–CO–C$_6$H$_5$	3·0		>0·80
3160	–N(cyclohexyl)–CO–C$_6$H$_5$*	>4·0		Inactive
3630	–N(CO–C$_6$H$_5$)–[CH$_2$]$_5$–O–C$_6$H$_4$–NH$_2$–*p*	1·5		>0·40
3727	–N(OH)–CO–C$_6$H$_5$	>4·0		Inactive
3728	–N(O–CH$_2$–C$_6$H$_5$)–CO–C$_6$H$_5$	>4·0		0·80
3301		1·5		Inactive
3414		1·5		0·20
4265		>4·0		>0·20
4301		>4·0		Inactive

TABLE VIII

THE OCULAR EFFECTS OF M&B2948A AND M&B3002 IN CATS

The asterisk refers to the methanesulphonate of M&B2948A, *ca.* 0·1% soluble in water; †, the methanesulphonate of M&B3002, *ca.* 3·0% soluble in water; ‡, administered twice daily; §, three of these cats had previously had one dose of either 1·3 or 0·4 g./kg., and had not shown ocular effects; ||, see Table I for formula. Values in brackets refer to numbers of cats.

M&B No.	Daily Oral Dose (g./kg.)	No. of Doses	No. Showing Ocular Effects
2948A	1·0 (10)	3–4	1
	0·8 (2) and 0·4 (2)	1	1 (given 0·4 g./kg.)
	0·5 (13)	3–4	3
	0·25 (10)	3–4	2
	0·125 (10) and 0·08 (10)	4	0
2948*	1·6 (1) and 1·3 (1)	1	0
	0·8 (11)§	1	5
	0·5–0·4 (16)	4–20	3 (1 after 4, 1 after 5, and 1 after 20 doses)
	0·4 (7), 0·25 (2), and 0·15 (2)	1	0
	0·04 (2)	6‡	0
3002	1·9 (1)	1	1
	1·0 (10)	2–4	4
	0·5 (10)	2–4	2
	0·45–0·40 (14)	4–20	5 (2 after 4, 1 after 14,‡ and 2 after 20 doses)
	0·4 (6)	1	1
	0·25 (10), 0·125 (10) and 0·08 (10)	3–4	0
	0·04 (1)	6‡	0
3002A†	0·4 (4)	1	3
	0·04 (1)	6‡	0
3637||	0·8 (8)	3–4	2

sp.) infected with *S. mansoni* were given by mouth 0.4 g./kg. of M&B2948A daily for four days. Two other infected rhesus monkeys were kept as controls. All the treated monkeys continued to pass viable eggs for 23 days after the end of treatment. One of the rhesus monkeys was then killed and examined; the remaining monkeys continued to pass eggs for at least 45 days after treatment. The monkey which was killed had four pairs of worms in the mesenteric veins and one male worm in the liver. No signs of ensheathed worms were found in liver-crush preparations. The drug was inactive, therefore, although similar treatment was highly effective in mice.

M&B2948A was active against *S. mansoni* in hamsters after four daily doses of 0.4 g./kg. This treatment killed a total of 57 out of 60 worms in three hamsters.

With M&B3002, the effect of various dosing schedules was investigated in mice. A single dose, doses once daily for two days, twice daily for two days, once daily for four days, twice daily for four days, and once daily for eight days, giving total doses of 0.8 g./kg., were employed in each instance. There was no significant difference between the effects produced by these treatments.

The rate of action of M&B3002 was studied after a single oral dose of 1.6 g./kg. After 24 hr. the worms had begun to drift back to the liver, although about 20% of them (a total of 25 pairs in 12 mice) were still in the mesenteric veins. The males and females in the portal vein and liver were beginning to separate. After 48 hr. only 3% (a total of 3 pairs in 11 mice) of the schistosomes were found in the mesenteric veins, and 4% in the portal vein. Ensheathment had already begun although none of the worms was completely ensheathed, their ends being free and mobile (Standen, 1955). Seven days after dosing many of them were completely ensheathed. The ratio of males to females on this occasion was 9:1, which may have been due to the more rapid ensheathment of the females or to the fact that they were missed because they had become so small and degenerate. All the females which were seen were paired in the mesenteric veins, but only 11% of the males (a total of three in five mice) were in this site, the rest being in the liver. Fourteen days after treatment the sex ratio was 1:1, as it had been at the beginning of the experiment. Half of the living worms were found in the mesenteric veins and half in the portal vein, but the average number of schistosomes/mouse was only 0.25 pairs in eight mice compared with 19.8 pairs for the 12 untreated controls examined at the beginning of the experiment. About 98% of the worms had, therefore, been killed by the drug.

The effect of M&B3002 was also investigated on worms at various stages of development. Five groups of mice at 4 to 5 weeks, 5 to 6 weeks, 6 to 7 weeks, 7 to 8 weeks, and 8 to 9 weeks respectively after infection were all given 0.4 g./kg. daily for four days. They were examined two weeks after treatment or seven to eight weeks after infection, whichever was the later. The results showed that M&B3002 was inactive against worms 4 to 5 weeks old, and that the activity increased from 5 to 6 to 8 to 9 weeks. Another experiment in which mice were treated daily for five days and infected between the second and third doses demonstrated that M&B3002 had no prophylactic activity.

A dose of 0.4 g./kg. of M&B3002 daily for four days was found, like M&B2948A, to be active against *S. mansoni* in hamsters. A total of 61 out of 66 worms in four hamsters were killed.

General Toxicology.—A single oral dose of 4.0 g./kg. of M&B2948A was not lethal to mice, and 4.0 g./kg. of its methanesulphonate was not lethal to mice, rats, and rabbits. The same dose

of M&B3002 was not lethal to mice and rabbits, but caused some deaths in rats, the LD50 in this species being about 3.0 g./kg. (five rats/dose).

Four daily doses of 1.0 g./kg. of M&B2948A were not lethal to cats and monkeys (Tables VIII and IX) although the LD50 in mice was 1.02 g./kg. (Table IV). Guinea-pigs tolerated four daily doses of 0.4 g./kg., but higher doses were not given.

TABLE IX

THE TOXIC EFFECTS OF M&B2948A AND M&B3002 IN RHESUS MONKEYS

The drug marked by an asterisk was the methanesulphonate of M&B2948A, *ca.* 0·1% soluble in water.

M&B No.	Daily Oral Dose (g./kg.)	No. of Doses	No. of Monkeys Treated	No. Showing Ataxia	No. Showing Ocular Effects
2948A	1·0	4	8	0	0
	0·5	4	8	0	0
2948*	1·0	5 and 20	2	0	0
3002	1·0	4	10	9	0
	0·5	4	10	2	0

Four daily doses of 1.0 g./kg. of M&B3002 were not lethal to cats (Table VIII) although they killed seven out of nine rats. The LD50 in mice was 0.88 g./kg. (Table II) and in guinea-pigs about 0.2 g./kg. (five out of nine being killed by this dose). In monkeys (Table IX), four daily doses of 1.0 g./kg. and 0.5 g./kg. produced ataxia and general weakness so that the affected monkeys had to be killed.

M&B2948A and M&B3002 were administered in the diet for 12 days to young rats (five males and five females/group) weighing 50 to 60 g. (Table X). There was some depression of weight gain with 1% M&B2948A and with 0.4% M&B3002, but growth rates returned to normal as soon as non-medicated diet was substituted. Lucanthone retarded the growth rate at 0.12%, but was without effect at 0.02%.

TABLE X

EFFECT OF M&B2948A AND M&B3002 ON GROWTH RATE IN RATS

The period marked by the asterisk is the treatment period; †, the methanesulphonate of M&B2948A.

Compound	Concentration in Diet (%)	Average Weight Gain (g.)	
		Days 1–12*	Days 13–22
M&B2948A ..	1·0	7·5	30
Lucanthone ..	0·02	34	25
Controls	—	39	25
M&B3002 ..	0·4	5	35
M&B2948† ..	0·4	25	30
Controls	—	32	30
Lucanthone ..	0·12	−1	40
,, ..	0·05	20	35
Controls	—	31	27

Vomiting was observed in 10 out of 57 cats given M&B2948A, in 24 out of 70 given M&B3002 and in 8 out of 11 dogs given the methanesulphonate of M&B2948A.

Two monkeys were given 0.25 g./kg. of the methanesulphonate of M&B3002 intraperitoneally twice daily. One of these was killed on the seventh day of the experiment, having been treated for five days. It had become listless and subdued by the third day and this condition increased in severity until the animal was moribund. No effect on the eyes was observed and no macroscopic abnormalities were seen *post mortem*. The second monkey was treated for only three days when it had to be killed. Signs of toxicity included listlessness, partial paralysis of the limbs, and vomiting. Again no ocular effects were observed.

No abnormalities were seen in the blood pictures or blood urea concentrations of three guinea-pigs given 0.4 g./kg. of M&B2948A or 0.1 g./kg. of M&B3002 five times a week for three weeks. Histological studies carried out by Professor N. Ashton showed, however, that both compounds were capable of producing in guinea-pigs some focal necrosis in the liver and kidneys, and inflammatory changes in the lungs, although the degree of pathological change was variable. The principal feature was the presence of hyaline material in the glomerular capillaries and in the lungs, giving a positive reaction to periodic acid-Schiff reagent for the detection of glycogen.

With M&B3002, death, when observed, was preceded by prostration, and the guinea-pigs exhibited weakness, particularly of the hind limbs; breathing was shallow and the body temperature was 35° or less. The actual cause of death remains unknown.

Ocular Effects in Cats.—Only a small proportion of the cats treated with M&B2948A or M&B3002 showed visual impairment (Table VIII).

The eyes of two cats given 20 doses each of 0.4 g./kg. of the methanesulphonate of M&B2948A and killed 23 days after the last dose were examined histologically by Professor N. Ashton, who found no abnormalities in one cat, and the following picture in one of the eyes of the other animal, the second eye being normal. "Immediately to one side of the disc there is a sharply circumscribed area in which the outer layers of the retina have been destroyed and become gliosed. Elsewhere the retina shows slight rosette formation—but no degeneration or pigmentary disturbance. Optic nerve normal."

A third cat which had been given 14 similar doses and killed 48 days after the end of treatment showed "acute-angle retinal folds in all sections of both eyes. At the base of some of these folds there was a slight proliferation of pigment epithelial cells." Professor Ashton said that this cat appeared "to have recovered from very slight early changes."

A fourth cat was given five daily doses of 0.5 g./kg. of the same compound, and subsequently its behaviour indicated gross impairment of vision. It was killed two days after the last dose. Professor Ashton's report on this cat was: "Right eye: The only abnormality in this eye is to be seen in the retina, which shows almost total destruction of the rod and cone layer with acute-angled folding of the retina in the region of the disc. The pigment layer is normal posteriorly but has proliferated anteriorly to form clumps of cells containing aggregations of pigment. Special staining (PAS, Weils, Holmes, and phosphotungstic stains) shows that the remainder of the retina, ganglion cells, and optic nerve are unaffected. Left eye: Shows an exactly similar picture."

A fifth cat given 20 daily doses of 0.45 g./kg. in the same experiment was killed 106 days after the last dose and the report for both eyes was: "There is a patchy loss of the rod and cone layer but nowhere is the destruction complete. In the affected regions there is considerable pigmentary disturbance and occasional angulation of the retina. Aggregation and migration of pigment are particularly marked at the equator where a few colloid bodies are seen. The inner layers of the retina and optic nerve are normal."

Nothing abnormal was found in the eyes of two cats given 0.04 g./kg. of the methanesulphonate of M&B2948A twice daily for three days.

Professor Ashton also examined the eyes of three cats which had received M&B3002 (0.4 g./kg. × 20; 0.4 g./kg. × 14; 0.04 g./kg. × 6) and of one which had had the methanesulphonate of this base (0.04 g./kg. × 6). He found no changes that could be ascribed to the toxic action of the drug, although one of these animals (M&B3002 0.4 g./kg. × 14) exhibited mydriasis.

Absorption and Excretion.—The results of these studies are given in Tables XI and XII. The values were obtained from pooled samples from 15 mice or 3 rats for blood, and 30 mice or 6 rats for urine and faeces. The blood concentration of M&B2948A in rats rose to 6 μg./ml. at 2 hr., remained there until 4 hr., and was undetectable (less than 1 to 2 μg./ml.) at 6 hr. In both species most of the drug was excreted in the first 24 hr. and very little was recovered after that period.

TABLE XI

BLOOD CONCENTRATIONS OF M&B2948A AND M&B3002

Blood concentrations refer to total drug, with free drug concentrations in brackets.

Animal	M&B No.	Dose (g./kg.)	Blood Concentration (μg./ml.) 1 hr.	4 hr.	8 hr.	24 hr.
Mice	2948A	0·25	8·0 (0)	2·0 (0)	1·5 (0)	0
	3002	0·25	30·5 (11·0)	23·0 (1·0)	9·0 (0)	0
Rats	2948A	0·25	0	6·0 (0)	0	0
	3002	0·25	11·0 (0)	5·0 (3·0)	3·0 (0)	0

TABLE XII

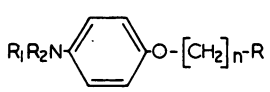
EXCRETION OF M&B2948A AND M&B3002

Animal	M&B No.	Dose (g./kg.)	Excretion Route	% Dose Excreted (72 hr. Period) Free	Total	Drug Recovery (%)
Mice	2948A	0·25	Urine	2·7	10·0	39·0
			Faeces	22·2	29·0	
	3002	0·25	Urine	5·2	20·6	50·5
			Faeces	28·3	29·9	
Rats	2948A	0·25	Urine	1·3	5·3	30·1
			Faeces	21·9	24·8	
	3002	0·25	Urine	3·1	26·0	46·4
			Faeces	18·0	20·4	

DISCUSSION

Screening Experiments

The active compounds discussed in this report are variants of the general formula which Collins



et al. (1958) considered to contain all the structural features essential for schistosomicidal activity in this chemical field. R_1 and R_2 can be hydrogen, lower alkyl, hydroxyalkyl, or alkoxyalkyl, and R, in addition to substituted or unsubstituted alkoxy, phenoxy, or phenyl moieties, as shown previously, may be an acylamido or imido group, although as pointed out in the introduction the parent amine (II) from which these acyl derivatives were developed was itself inactive. But whereas in the homologous series of diamines previously reported, activity extended from the propane (n=3) to the nonane (n=9) members, in a similar series of phthalimides (Table IV) only the pentane (n=5) to the nonane (n=9) homologues were active. In the above general formula, therefore, n must be 5 to 9 for the imides instead of 3 to 9 as it was for the diamines.

The inactivity of the phthalimides either without an aromatic amino group or with the amino group *ortho* or *meta* to the ether link confirms our previous finding that a *para* amino group is essential for activity.

The incidence of visual impairment in cats among the amides and imides was certainly much lower than it was among the compounds described by Collins *et al.* (1958), although the effect was not absent altogether.

M&B2948A and M&B3002

The inactivity of M&B2948A in monkeys was surprising in view of its performance in mice and hamsters, but this may be due to poor absorption or to a difference of metabolism in the different species, either of which reasons could also explain the lack of toxic signs in monkeys.

The rate of action of M&B3002 resembled that of 1,5-di(*p*-aminophenoxy)pentane dihydrochloride (M&B968A) which was found by Hill (1956) to be more rapid in its action than lucanthone (Miracil D). With the latter compound the worms had only just begun their shift to the liver seven days after treatment, and this migration was only complete after 14 days; also, ensheathment was not noticed until the 21st day, whereas with M&B3002 and M&B968A it was well advanced by the seventh and ninth days respectively. In a hitherto unpublished experiment a single intraperitoneal dose of 0.4 g./kg. of antimony potassium tartrate (tartar emetic) drove all the worms back to the liver within 6 hr., but they recovered and resumed their normal distribution within seven days. The action of antimony potassium tartrate was, therefore, much more rapid than that of the other compounds, but unlike them a single dose exerted only a temporary effect.

The poor activity of M&B3002 against immature worms is paralleled by that of the Miracils (A to D) which was recorded by Kikuth and Gönnert (1948). Bueding, Peters, Koletsky, and Moore (1953) have suggested that on reaching maturity the worms change from an oxidative to a glycolytic way of life; and since they reach maturity at about six weeks after infection, at which time M&B3002 began to be effective, it seemed possible that this compound acted by inhibiting glycolysis. One of us (H. W. R.) has indeed obtained biochemical evidence that compounds of this type do inhibit anaerobic glycolysis in schistosomes.

Toxic Effects.—M&B3002 was more toxic than M&B2948A in rats, guinea-pigs, and monkeys, an observation which in rats at least might be related to the better absorption of M&B3002, although in mice better absorption was accompanied by only a small increase in chronic toxicity which was probably not significant. M&B3002 was more toxic to guinea-pigs than to any of the other species, but it is not possible to say why, unless it was due to better absorption or to a difference in metabolism.

Turning to ocular effects, the methanesulphonate of M&B2948A in particular was shown to be capable of producing the gross destruction of the retina described by Ashton (1957) for 1,5-di(*p*-aminophenoxy)pentane dihydrochloride (M&B968A) although like M&B2948A and M&B3002 it was mydriatic in only a very small proportion of the cats. The methanesulphonate of M&B3002, the only really soluble compound tested, was more retinotoxic than the base, an observation which suggests that the lower incidence among the free bases was connected with their very low solubility, although several of the amines described by Collins *et al.* (1958) were toxic even when administered as insoluble bases.

Neither the benzamide M&B3002 nor the phthalimide M&B2948A produced any ocular effects in monkeys, even at a lethal dose, although 1,5-di(*p*-aminophenoxy)pentane dihydrochloride (M&B968A) was undoubtedly retinotoxic in single non-lethal oral doses of 0.4 g./kg. (unpublished observation). We therefore conclude that the amides and imides discussed here are less retinotoxic in monkeys and cats than the compounds described by Goodwin *et al.* (1957) and Collins *et al.* (1958).

Both M&B2948A and M&B3002 were examined for activity against *S. haematobium* in African schoolchildren, but as no difference could be detected between them in the preliminary trials (Alves and Harper, personal communication), either as regards tolerance or activity, M&B3002 was discontinued since it is slightly less accessible chemically than M&B2948A (Alves, Harper, and Hill, unpublished observations). Schneider and Sansarricq (1959) have found M&B2948A moderately effective against *S. haematobium* infections in Africans.

We wish to thank Professor N. Ashton for carrying out the pathological studies, our colleagues, Dr. J. N. Ashley, Dr. H. J. Barber and Dr. R. Wien, for their helpful discussions during this investigation, Miss N. E. Sirett for the preparation of some of the compounds, Mr. W. A. Freeman for carrying out the blood investigations and Mr. H. S. Alexander, Mr. I. Hodges and Mr. A. D. Speed for their technical assistance.

References

Ashton, N. (1957). *J. Path. Bact.*, **74**, 103.
Bueding, E., Peters, L., Koletsky, S., and Moore, D. V. (1953). *Brit. J. Pharmacol.*, **8**, 15.
Caldwell, A. G., and Standen, O. D. (1956). Ibid., **11**, 367.
Collins, R. F., Davis, M., Edge, N. D., and Hill, J. (1958). Ibid., **13**, 238.
Edge, N. D., Mason, D. F. J., Wien, R., and Ashton, N. (1956). *Nature, Lond.*, **178**, 806.
Goodwin, L. G., Richards, W. H. G., and Udall, V. (1957). *Brit. J. Pharmacol.*, **12**, 468.
Hill, J. (1956). *Ann. trop. Med. Parasit.*, **50**, 39.
Kikuth, W., and Gönnert, R. (1948). Ibid., **42**, 256.
Raison, C. G., and Standen, O. D. (1955). *Brit. J. Pharmacol.*, **10**, 191.
Rose, F. L., and Bevan, H. G. L. (1944). *Biochem. J.*, **38**, 116.
Schneider, J., and Sansarricq, H. (1959). *Méd. trop.*, **19**, 412.
Sorsby, A., and Nakajima, A. (1958). *Brit. J. Ophthal.*, **42**, 563.
Standen, O. D. (1955). *Trans. roy. Soc. trop. Med. Hyg.*, **49**, 416.